Department of Molecular Physiology
Division of Physical Fitness

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General Summary

Research activities in our division have been focused on the plasticity of skeletal muscle and preventive medicine against sarcopenia and metabolic syndrome in terms of exercise physiology.

Research Activities

Dysfunction of autophagy in old rats and unloading-induced sarcopenia

Unloading is known to induce sarcopenia in old rats with histological features characteristic of autophagy dysfunction. We have reported that intermittent resistance-exercise (IRE) ameliorates this sarcopenia. Here, we aimed to clarify the effects of IRE on autophagy in unloaded muscles of old rats. Old female F344 rats (2 years old, n = 21) were randomly divided into control, unloading, and unloading + IRE groups. Middle-aged rats (1 year old, n = 7) were used as an adult control group. Rats of the unloading and unloading + IRE groups had their hindlimbs unloaded by tail suspension. In the unloading + IRE group, IRE was performed 3 times per day for 10 minutes at 4-hour intervals in a dark period. The intervention period was 3 weeks. The mass of the lateral gastrocnemius was significantly lower in old control rats than in adult control rats. On the other hand, unloading-induced atrophy was more prominently observed in the soleus. The IRE ameliorated atrophy in both the lateral gastrocnemius and the soleus. In the red part of type I-rich lateral gastrocnemius, unloading selectively affected type I fibers disrupting myofibrils with a decrease in sarcomeric proteins, formation of the inclusion body, and accumulation of abnormal mitochondria. The IRE inhibited such type I fiber-specific disruptions. In type I-rich soleus, a significant decrease in myofibrilar protein was found to accompany increases in ubiquitinated proteins and ubiquitin ligase (Fbx32) and decreases in microtubule-associated protein light chain 3 (LC3)-II and mitochondrial calcium uniporter (MCU). The IRE increased myofibrillar protein, LC3-II, parkin, and MCU in the soleus. We conclude that functional alteration in autophagy and mitophagy might be involved in the effects of unloading and IRE prominently in type I fibers.

Increased muscle stiffness after eccentric contraction

We explored the cause of an increase in muscle stiffness after eccentric contraction (ECC) in rat plantaris muscle. During 0.3-second-long tetanic ECC elicited through tibial nerve stimulation in anesthetized 8-week-old male F344 rats, the plantaris muscle was stretched to the length for maximal isometric twitch tension (L0) from 0.9 L0. The H10, M10, and L10 groups received 10 ECCs elicited by 100, 75, and 50 Hz supramaximal stimulation, respectively. The L30 group received 30 ECC by 50 Hz stimulation. The ISO group

received submaximal isometric tetanus contraction by 100 Hz stimulation, and the CON group received no conditioning contraction. After the series of contractions, plantaris muscle was dissected for analysis with x-ray diffraction, electrophoresis, and the measurement of myofibrillar resting tension. The ECC in the H10 group significantly increased muscle stiffness. The deterioration of the microstructural sarcomere detected with x-ray diffraction patterns was slight in the ISO, L10, and L30 groups but was moderate in the M10 group and marked in the H10 group. The profiles of α-connectin, β-connectin, and myofibrillar resting tension did not differ significantly between the H10 group and the ISO group. On the basis of these results, we conclude that the increase in muscle stiffness in the H10 group is not due to any change in connectin. The increase in muscle stiffness might, therefore, represent a slight increase in the concentration of intracellular Ca^{2+} due to microscopic cellular injuries.

An x-ray diffraction study on in vivo skeletal muscle with maintained blood supply

X-ray diffraction is a fixation-free technique that enables the function and structure of living skeletal muscle to be evaluated. Its application to in vivo muscles with a maintained blood supply is expected to be of high potential in physiological studies of muscle adaptations, such as atrophy and hypertrophy. Each periodic repeat in sarcomeres gives rise to a specific series of reflections or layer-lines on an x-ray diffraction pattern. The intense reflection pairs (1,0 and 1,1 reflections) eminated bilaterally represent myofilament lattice reflecting volume change and the vital state of cells. As an initial attempt to apply this technique to the evaluation of in vivo muscle, we examined muscle states at very early days after denervation. A unilateral incision was made on the sciatic nerve of 6-month-old female ICR mice, with a sham operation on the other side. X-ray diffraction patterns from the extensor digitorum longus muscle of anesthetized mice were obtained 0, 0.5, 2, and 5 days after the operation at the BL-6A beamline for small angle X-ray scattering at the High Energy Accelerator Research Organization, Tsukuba. The patterns showing 1,0 and 1,1 reflections and several layer-lines representing helical arrangement of myoproteins were successfully obtained. Although muscles showed no signs of atrophy in tissue wet weight or the connectin/titin profile, 1,0 and 1,1 reflections of denerved muscles suggested cellular swelling in 0.5 day and subsequent partial deprivation of ATP in 5 days. We succeeded in obtaining x-ray diffraction patterns from in vivo muscle with a maintained blood supply. The pattern indicates very early effects of denervation on muscle cells.

Effect of exercise on the accumulation of ingested polyamine in various tissues of rats

Polyamines, such as putrescine, are polycation molecules indispensable for cell proliferation. Polyamines are also reported to modulate cardiac excitability and cell viability. We have recently found that exercise suppressed accumulation of ingested putrescine in the heart and serum of rats. In the present study, we examined the effects of exercise on the accumulation of ingested putrescine in other tissues. Six-week-old female Wistar rats were fed with drinking water containing 1 mg/ml putrescine for 9 weeks with or without a freely accessible wheel for spontaneous running. Their skeletal muscle, liver, lung, spleen, and fat were then dissected for polyamine content analysis with high-performance

liquid chromatography. Urine adsorbed on filter papers covering the cage bottom was also analyzed for polyamine metabolites. Exercise significantly suppressed the accumulation of ingested putrescine in all tissues tested. Although both putrescine ingestion and exercise induced no significant difference in tissue spermidine content, urinary spermidine showed a significant increase. Because spermidine is a metabolite of putrescine, we speculate that the ingested putrescine might be rapidly catabolized. With the possibility that exercise affects polyamine metabolism, more comprehensive analysis is required of metabolites with stable-isotope tracer analysis by mass spectrometry.